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Neomycin inhibits angiogenin-induced angiogenesis

(proliferation/nuclear translocation/inhibition)

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ABSTRACT **A class of angiogenesis inhibitor has emerged from our mechanistic study of the action of angiogenin, a potent angiogenic factor. Neomycin, an aminoglycoside antibiotic, inhibits nuclear translocation of human angiogenin in human endothelial cells, an essential step for angiogenin-induced angiogenesis. The phospholipase C-inhibiting activity of neomycin appears to be involved, because U-73122, another phospholipase C inhibitor, has a similar effect. In contrast, genistein, oxophenylarsine, and staurosporine, inhibitors of tyrosine kinase, phosphotyrosine phosphatase, and protein kinase C, respectively, do not inhibit nuclear translocation of angiogenin. Neomycin inhibits angiogenin-induced proliferation of human endothelial cells in a dose-dependent manner. At 50 μM, neomycin abolishes angiogenin-induced proliferation but does not affect the basal level of proliferation and cell viability. Other aminoglycoside antibiotics, including gentamicin, streptomycin, kanamycin, amikacin, and paromomycin, have no effect on angiogenin-induced cell proliferation. Most importantly, neomycin completely inhibits angiogenin-induced angiogenesis in the chicken chorioallantoic membrane at a dose as low as 20 ng per egg. These results suggest that neomycin and its analogs are a class of agents that may be developed for anti-angiogenin therapy.**

Angiogenin, a potent inducer of neovascularization (1), is an angiogenic protein first isolated from tumor-conditioned culture medium (2) in the course of a search for tumor angiogenesis agents. This search was based on the hypothesis that tumors will not grow beyond a minuscule size unless they are supplied with new blood vessels to provide nutrients and facilitate gas exchange (3). They elicit the formation of these blood vessels by secreting angiogenesis factors. Exogenous angiogenin has been demonstrated to induce the formation of new blood vessels in the chicken chorioallantoic membrane (CAM) and in the cornea and meniscus of the knee of rabbits (2, 4). Anti-angiogenin mAbs (5) have been shown to prevent the growth of human colon, lung, and fibroid tumor cells implanted subcutaneously into athymic mice (5, 6).

Structure/function studies have indicated that the weak but characteristic ribonucleolytic activity of angiogenin is essential for its angiogenic activity. Anti-angiogenin inhibitors have been identified based on their ability to block this ribonucleolytic activity. Thus, the C-terminal peptide of angiogenin, the ribonuclease inhibitor from human placenta, and, more recently, a deoxynucleotide aptamer obtained by exponential enrichment have been shown to inhibit both the ribonucleolytic and angiogenic activities (1, 7).

Interaction of angiogenin with endothelial cells is another area of research for the advancement of anti-angiogenin therapy. Angiogenin binds to actin (8, 9) and to a 170-kDa putative receptor (10) that are expressed on the surface of endothelial cells growing in dense and sparse cultures, respectively. Binding of angiogenin to endothelial cells activates phospholipase C (PLC) (11), cell migration and invasion (12), proliferation (10), and differentiation (13). A cell binding site has been identified that encompasses residues not involved in catalysis but essential for angiogenesis. Intervention of the interaction between angiogenin and its target cells has also been used to inhibit angiogenesis. For instance, both actin and an anti-actin antibody completely inhibit angiogenin-induced angiogenesis on the CAM (9). Moreover, actin has been shown to prevent the growth of human tumor cells in nude mice (5).

A striking feature of angiogenin is that it normally circulates in human plasma at a concentration of 250–360 ng/ml (14). Plasma angiogenin may promote wound healing when it becomes extravascular, e.g., through trauma. However, circulating angiogenin would be a major obstacle for anti-angiogenin therapies that would attempt to neutralize it to suppress unwanted angiogenesis. Moreover, angiogenin mRNA and protein are elevated in tissues and cells of patients with a variety of tumors (15, 16) and this would make neutralization even more difficult. Thus, inhibitors that target the angiogenin receptor might circumvent the potential problems associated with circulating angiogenin and might be more suitable for anti-angiogenin therapy.

Angiogenin is internalized by endothelial cells and translocated to the nucleus by a process that is independent of lysosomes and microtubules (17–19) but is necessary for angiogenesis. Angiogenin variants with an altered nuclear localization sequence have full enzymatic activity and capacity to bind to the endothelial cell surface, but do not undergo nuclear translocation and are not angiogenic on the CAM (17). Because translocation of angiogenin from the exterior of the cell to the nucleus is a multistep process that involves internalization, transport across the cytoplasm, and entrance into the nucleus, it provides several opportunities to control angiogenin activity. Interruption of any one of these steps could result in inactivation of angiogenin-induced neovascularization. More importantly, drugs targeted on the nuclear translocation process should not encounter problems with circulating angiogenin.

This study extends our investigation of the mechanism of nuclear translocation by examining the role of PLC activation in this process. We find that neomycin, an aminoglycoside antibiotic and a known PLC inhibitor, potently inhibits both nuclear translocation of angiogenin and angiogenin-induced cell proliferation and angiogenesis. The results suggest that neomycin and its analogs offer potential as a new class of anti-angiogenin agents that might augment the agents available for the clinical treatment of angiogenesis-related diseases.

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Abbreviations: CAM, chorioallantoic membrane; HE-SFM, human endothelial serum-free medium; HUVE, human umbilical vein endothelial; PLC, phospholipase C.

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MATERIALS AND METHODS

Materials. Human angiogenin (Met-1) was a recombinant product from an *Escherichia coli* expression system (20). Fertilized chicken eggs were from SPAFAS (Norwich, CT). Neomycin, amikacin, gentamicin, kanamycin, paromomycin, streptomycin, penicillin, amoxicillin, bacitracin, erythromycin, staurosporine, oxophenylarsine, yeast tRNA, and ribonuclease-free BSA were from Sigma; U-73122 and U-73343 were from Calbiochem; genistein was from Indofine Chemical Company (Somerville, NJ); basic fibroblast growth factor was from Promega; human endothelial serum-free medium (HE-SFM) was from GIBCO/BRL-Life Technologies; fetal bovine serum was from HyClone; Excellulose GF-5 desalting columns and Iodo-Beads iodination reagents were from Pierce; [*methyl*-^{3}H]thymidine (6.7 Ci/mmol; 1 Ci = 37 GBq) and Na^{125}I (17.4 Ci/mg) were from DuPont/NEN.

Cell Culture. Human umbilical vein endothelial (HUVE) cells were purchased from Cell Systems (Kirkland, WA). The cells were cultured in HE-SFM supplemented with 10% fetal bovine serum and 10 ng/ml basic fibroblast growth factor at 37°C under humidified 5% CO_2 and were split 1:3 for subculture. Cells from passages 5 to 12 inclusive were used for all experiments. Cell numbers were determined with a Coulter Counter, and cell viability was measured by the trypan blue dye exclusion assay.

Iodination of Angiogenin. ^{125}I-labeled angiogenin was prepared with the use of Iodo-Beads as described previously (10). The specific activity of ^{125}I-angiogenin used in the experiments ranged from 1 to 2×10^6 cpm/μg.

Nuclear Translocation. HUVE cells were seeded at 5×10^3/cm^2 in 35-mm dishes and cultured in HE-SFM supplemented with 20 ng/ml basic fibroblast growth factor at 37°C under humidified 5% CO_2 for 24 hr. The cells were washed three times with prewarmed (37°C) HE-SFM and incubated with ^{125}I-angiogenin (1 μg/ml) at 37°C for 30 min. Two procedures were used to examine the effect of inhibitors on nuclear translocation. The first was to premix the inhibitors with ^{125}I-angiogenin and adjust the sample volume to 10 μl with HE-SFM before addition to the cells. The second was to pretreat the cells in HE-SFM with the inhibitors for 10 to 30 min before ^{125}I-angiogenin was added to the cells. After incubation, the dishes were cooled at 4°C for 10 min and the medium was removed. The cells were washed three times with cold PBS, detached by scraping, and centrifuged at $800 \times g$ for 5 min. The cells were washed once with PBS and lysed by 0.5% Triton X-100 in PBS. The nuclear fraction was isolated by centrifugation at $1200 \times g$ for 5 min. Radioactivity was determined with a γ counter.

Cell Proliferation. HUVE cells were seeded at 4×10^3 cells per cm^2 in attachment factor (Cell Systems)-coated 35-mm dishes in HE-SFM, and incubated with 1 μg/ml angiogenin in the presence or absence of inhibitors at 37°C for 48 hr. Cells were detached by trypsinization and cell numbers were determined with a Coulter Counter.

Ribonucleolytic Assay. The effect of neomycin on the ribonucleolytic activity of angiogenin was examined with yeast tRNA as the substrate. Angiogenin, or its mixture with neomycin, was added to an assay mixture containing 0.6 mg of yeast tRNA, 30 μg of ribonuclease-free BSA, 30 mM Hepes (pH 6.8), and 30 mM NaCl in a final volume of 300 μl. After incubation for 2 hr at 37°C, 700 μl of 3.4% ice-cold perchloric acid was added, the mixture was vortexed, kept on ice for 10 min, and centrifuged at $15{,}000 \times g$ for 10 min at 4°C. The absorbance of the supernatants was measured at 260 nm.

RESULTS

Neomycin Inhibits Nuclear Translocation of Angiogenin in HUVE Cells. Exogenously added angiogenin is rapidly taken up and translocated to the nucleus of proliferating endothelial cells (17). The mechanism of translocation is not yet known; but it seems to be energy and temperature dependent, suggesting that it involves receptor-mediated endocytosis (17). Angiogenin also induces DNA synthesis and proliferation of sparsely cultured human endothelial cells (10). Therefore, we investigated the relationship of signal transduction and nuclear translocation by examining the effect of specific inhibitors of enzymes thought to be involved in the signal transduction process on the nuclear translocation of angiogenin in HUVE cells. As shown in Table 1, genistein and oxophenylarsine, inhibitors of tyrosine kinase and phosphotyrosine phosphatase (21), respectively, have no effect on nuclear translocation of ^{125}I-angiogenin. Staurosporine, an inhibitor of protein kinase C, at its optimal concentration of 100 nM (21), was only marginally inhibitory. However, 100 μM neomycin, an aminoglycoside antibiotic and a PLC inhibitor (22, 23), decreased the amount of ^{125}I-angiogenin accumulated in the cell nucleus by up to 60% after 30 min incubation. Another inhibitor of PLC, U-73122, also significantly inhibited nuclear translocation of ^{125}I-angiogenin (30% inhibition at 10 μM), whereas, its inactive analog, U-73343, had no effect. These data show that inhibitors of PLC inhibit nuclear translocation of angiogenin in HUVE cells, implying that PLC activity is required for translocation.

Neomycin inhibits nuclear translocation of angiogenin in a dose-dependent manner (Fig. 1*A*). Increasing concentration of neomycin progressively decrease the amount of nuclear ^{125}I-angiogenin from 3090 ± 260 cpm in the control to 420 ± 100 cpm in the presence of 500 μM inhibitor. The inhibition is not linear. At 10 μM, nuclear translocation is already inhibited by 42%. Increasing the concentration to 200 μM increases inhibition only by another 23%. Nuclear translocation cannot be abolished completely by neomycin. At 500 μM, the amount of ^{125}I-angiogenin that accumulates in the nucleus is 14% of that in the control.

Neomycin Inhibits Angiogenin-Induced Cell Proliferation. Exogenous angiogenin stimulates DNA synthesis and cell proliferation of sparsely cultured human endothelial cells (10). Because neomycin inhibits nuclear translocation of angiogenin, a necessary step for angiogenesis, we examined its effect on angiogenin-induced cell proliferation. When cells were cultured under the conditions described, essentially all were recovered after 48 hr in the absence of angiogenin and neomycin. In the presence of 1 μg/ml angiogenin, cell number after 48 hr increased by 35%. Neomycin alone neither induced nor inhibited cell proliferation. However, it inhibited angiogenin-induced cell proliferation in a dose-dependent but nonlinear manner. Thus, 5 μM neomycin already inhibited the proliferative activity of angiogenin by 49% (Fig. 1*B*). Increasing the neomycin concentration to 25 μM inhibited angiogenin-induced cell proliferation by 69%. Abolishment was achieved at 50 μM neomycin.

Table 1. Inhibition of nuclear translocation of angiogenin

Inhibitor	Nuclear ^{125}I-angiogenin, cpm	% inhibition
Control	3,090 ± 260	0
Genistein (100 μM)	3,300 ± 170	0
Oxophenylarsine (10 μM)	3,040 ± 70	0
Staurosporine (100 nM)	2,710 ± 70	12
Neomycin (100 μM)	1,230 ± 60	60
U-73122 (10 μM)	2,140 ± 30	31
U-73343 (10 μM)	2,890 ± 100	6

HUVE cells, 50,000 per 35-mm dish, were treated with inhibitors at 37°C for 30 min. ^{125}I-angiogenin was added to a final concentration of 1 μg/ml and incubated at 37°C for 30 min. Nuclear fractions were isolated and radioactivities were determined.

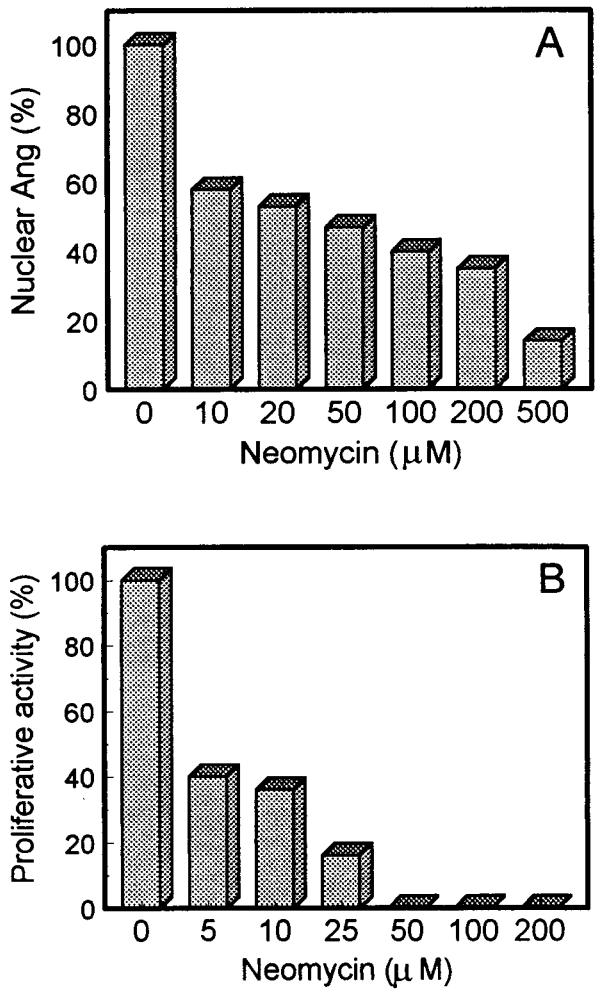

FIG. 1. Neomycin inhibits nuclear translocation of ^{125}I-angiogenin in (*A*) and angiogenin-induced proliferation of (*B*) HUVE cells. (*A*) HUVE cells were cultured at 50,000 cells per 35-mm dish and were treated with neomycin at the concentrations indicated. ^{125}I-angiogenin was added to a final concentration of 1 μg/ml and incubated at 37°C for 30 min. Nuclear fractions were isolated and radioactivities were determined. In a typical experiment, nuclear ^{125}I-angiogenin amounts in the presence of 0, 10, 20, 50, 100, 200, and 500 μM neomycin were 3,090 ± 260, 1,790 ± 50, 1,630 ± 70, 1,440 ± 300, 1,230 ± 60, 1,090 ± 50, and 420 ± 100 cpm, respectively. Data shown are percentages relative to the control. (*B*) HUVE cells were cultured at 40,000 cells per 35-mm dish and stimulated with 1 μg/ml angiogenin in the absence or presence of neomycin at the concentrations indicated at 37°C for 48 hr. In a representative experiment, cell numbers in the absence of angiogenin and neomycin or in the presence of 100 μM neomycin were 39,400 ± 300, and 39,200 ± 800, respectively. Cell numbers after stimulation with 1 μg/ml angiogenin in the presence of 0, 5, 10, 25, 50, 100, and 200 μM neomycin, were 53,200 ± 200, 46,500 ± 400, 46,000 ± 600, 43,800 ± 1600, 39,300 ± 800, 39,500 ± 1100, and 35,200 ± 1000, respectively. Increase of cell number stimulated by angiogenin at each neomycin concentration was calculated and compared with that in the absence of neomycin, which was defined as 100% proliferative activity.

Neomycin Inhibits the Angiogenic Activity of Angiogenin. The capacity of neomycin to inhibit angiogenin-induced angiogenesis was tested in the CAM assay. Neomycin itself at concentrations ranging from ≈5–50 μM (20–200 ng in the 5 μl volume applied) does not induce angiogenesis, nor does it cause necrosis or any other visible adverse effect on the chicken embryo (Table 2). Angiogenin alone at 10 ng induced a positive response in 55% of the eggs, consistent with previous results (2). Neomycin, 4 ng, decreased the number of positive eggs induced by 10 ng angiogenin from 55 to 40% and 20 ng decreased it to 20%, the same percentage as obtained with a water control. Thus, a dose of 20 ng of neomycin per egg or higher completely inhibits angiogenin-induced angiogenesis.

Table 2. Effect of neomycin on the activity of angiogenin in the CAM assay

Sample	Total eggs	% positive
Angiogenin (10 ng)	76	55
Neomycin (20 ng)	50	20
Neomycin (200 ng)	29	21
Angiogenin (10 ng) + neomycin (4 ng)	40	40
Angiogenin (10 ng) + neomycin (20 ng)	40	20
Angiogenin (10 ng) + neomycin (200 ng)	20	25
Water	128	20

Angiogenesis was measured on the CAM as described (2). Growth of blood vessels was observed microscopically and recorded as either positive or negative after 48 hr of incubation. Data were combined from multiple sets of experiments each using between 10 and 20 eggs. A positive response in ≈20% of the eggs is usually observed in water control and is considered as background angiogenesis which is not affected by inhibitors.

Effect of Neomycin on the Ribonucleolytic Activity of Angiogenin. The effect of neomycin on the ribonucleolytic activity of angiogenin, another property essential for angiogenesis, was examined with yeast tRNA as the substrate. The ribonucleolytic activity of angiogenin in the presence of 5, 10, and 50 μM neomycin was 87%, 105%, and 88% of that of the control, respectively. At higher concentrations, neomycin forms precipitates with tRNA. These results show that neomycin does not inhibit the cleavage of yeast tRNA by angiogenin even at a concentration of 50 μM when the proliferative and angiogenic activities are already abolished. These data suggest that the inhibitory activity of neomycin on angiogenin-induced blood vessel formation is attributable not to its effect on the ribonucleolytic activity of angiogenin, but rather to its inhibition of nuclear translocation of angiogenin in endothelial cells and/or its inhibition of angiogenin-induced cell proliferation.

Effect of Other Aminoglycoside Antibiotics. Other members of the aminoglycoside antibiotic family were also examined for their capacity to inhibit angiogenin-induced proliferation. None of the commonly used aminoglycosides—streptomycin, kanamycin, gentamicin, and amikacin—inhibit angiogenin-induced cell proliferation (Table 3). Significantly, paromomycin, which differs from neomycin only at position 6 of the glucose ring, does not inhibit angiogenin-induced cell proliferation. Thus, a single substitution of -NH_2 by -OH renders the aminoglycoside completely inactive as an anti-angiogenin agent. Preliminary data with the CAM assay indicate that

Table 3. Effects of aminoglycoside antibiotics on angiogenin-induced cell proliferation

Aminoglycoside (100 μM)	Angiogenin (1 μg/ml)	Cell number	%*
None	−	52,000 ± 100	
	+	62,500 ± 100	120
Neomycin	−	52,700 ± 700	
	+	53,400 ± 1,900	101
Amikacin	−	51,700 ± 200	
	+	61,000 ± 400	118
Streptomycin	−	51,900 ± 1,300	
	+	59,900 ± 900	115
Kanamycin	−	48,800 ± 400	
	+	58,900 ± 200	121
Gentamicin	−	45,700 ± 500	
	+	55,700 ± 900	121
Paromomycin	−	50,900 ± 500	
	+	58,900 ± 400	116

*Percent of cell numbers in the presence of 1 μg/ml angiogenin relative to the corresponding control.

amikacin and streptomycin do not inhibit angiogenin-induced angiogenesis. Among the aminoglycoside antibiotics, only neomycin is known as PLC inhibitor. Thus, these data further suggest that the PLC-inhibiting activity of neomycin is involved in its inhibition of angiogenesis

DISCUSSION

Growing evidence has demonstrated that control and modulation of angiogenic activity is important for the development, repair, and growth of normal and abnormal tissues. Angiogenin antagonists, including mAbs and its soluble binding protein, actin, prevent the establishment and growth of human tumor cells in athymic mice (5, 6). Histological data indicate that these antagonists reduce or diminish the tumor tissue neovasculature (5). These results suggest that angiogenin is involved in the initiation of the angiogenesis that is critical for the growth of tumors. Anti-angiogenin therapy could therefore play an important role in the clinical treatment of a number of diseases including cancer, arthritis, psoriasis, and diabetic retinopathy.

Among the proteins established to be angiogenic (24), angiogenin is different because it is a member of the ribonuclease superfamily and was the first to be identified as a tumor-derived angiogenesis factor and subsequently shown to be a normal constituent of plasma. Its relatively high concentration in plasma may, on the one hand, provide for prompt repair of blood vessel damage caused by a variety of physical, chemical, and pathological mechanisms such as trauma, oxidative stress, and ischemia. The activity of angiogenin circulating in plasma is regulated tightly to preclude unwanted rampant angiogenesis. Thus, it is inactivated by elastase in a process that is enhanced by plasminogen, and could thereby provide a regulatory system. On the other hand, the high concentration of circulating angiogenin (250–360 ng/ml) (14) and its fast turnover rate ($t_{1/2} < 5$ min) (25) make attempts to inhibit angiogenin activity by neutralizing the protein problematic. For this reason, research on the cell biology of angiogenin and its mechanism of action has become a more attractive approach that might lead to the design of inhibitors targeting its intracellular functions.

Nuclear translocation of angiogenin is necessary for its angiogenic activity, and because it is a multistep process, it affords a range of opportunities to identify possible inhibitors. As reported here, neomycin inhibits this process and, consequently, is an efficient inhibitor of angiogenin-induced angiogenesis.

Neomycin, an aminoglycoside, is an antibiotic that inhibits translation by binding to the small subunit of prokaryotic ribosomes, thereby causing misreading of mRNA. Unlike its structurally related compound, geneticin (G418; which is known to bind to 80S ribosomes, thus blocking protein synthesis in eukaryotic cells and thereby becoming a useful, selective marker for gene transfection; see ref. 26), neomycin does not bind to eukaryotic ribosomes. We have not observed cytotoxicity of neomycin to HUVE cells up to 200 μM. The other members of the aminoglycoside antibiotic family that we have examined, including amikacin, streptomycin, kanamycin, gentamicin, and paromomycin are also not toxic to HUVE cells.

Among these aminoglycoside antibiotics, neomycin is the only one that shows inhibitory activity to angiogenin-induced cell proliferation. It is noteworthy that the structurally very similar analog, paromomycin, does not exhibit any inhibitory activity at all. It is therefore apparent that the amino group on the carbon 6 of the glucose ring plays an important role in this inhibition of angiogenin-induced cell proliferation and angiogenesis.

Inhibition of nuclear translocation of angiogenin by neomycin is at least one of the reasons that leads to the inhibition of angiogenin-induced cell proliferation and angiogenesis. The concentrations required to inhibit nuclear translocation and cell proliferation by 50% are ≈50 and 10 μM, respectively. It is therefore possible that some other functional aspects of neomycin, which remain to be investigated, may also contribute to its anti-angiogenesis activity. It is known that in hair cells of the outer ear, neomycin binds to phosphatidylinositol 4,5-bisphosphate, thereby disturbing the synthesis of inositol phosphates (27). A blockage of the inositol phosphate transduction system could be a common factor underlying aminoglycoside-induced ototoxicity. However, the concentration of neomycin required to reach significant ototoxicity is much higher than that required to inhibit angiogenin-induced angiogenesis. Thus, for example, addition of 30 mM neomycin to the outer hair cells does not alter the basal level of inositol phosphate synthesis, whereas, 20 ng of neomycin per egg completely inhibits angiogenin-induced formation of new blood vessels. Nevertheless, binding of neomycin to phosphatidylinositol 4,5-bisphosphate could also contribute to its anti-angiogenin activity.

Addition of antibiotics to the culture medium of HUVE cells decreases the amount of von Willebrand factor deposited in the cellular matrix (28). Among the 14 antibiotics examined, only amphotericin, penicillin, streptomycin, and rifamycin had no significant influence on the composition and reactivity of the endothelial cell matrix. All the other antibiotics decrease the amount of von Willebrand factor in the matrix to various extents. Neomycin, at 100 μg/ml (≈100 μM), reduced the amount of von Willebrand factor in the matrix by 20%. Because the composition and property of extracellular matrix can significantly influence the function and metabolism of the cells, it is possible that a change of cell matrix by neomycin may also contribute to the anti-angiogenin activity. However, other antibiotics such as gentamicin and kanamycin also decrease the amount of von Willebrand factor in the matrix but are not anti-angiogenic. Therefore, change of matrix composition alone is not sufficient for the anti-angiogenin activity of neomycin.

Nuclear translocation of angiogenin in endothelial cells is thought to involve receptor-mediated endocytosis (17). However, binding of angiogenin to its surface receptor and the subsequent internalization do not seem to be inhibited by neomycin. Actually, neomycin induces a concomitant increase of cytosolic ^{125}I-angiogenin with the decrease of nuclear ^{125}I-angiogenin (data not shown). If the PLC-inhibiting activity of neomycin is responsible for the inhibition of nuclear translocation of angiogenin, these results suggest that PLC activity is required for the steps subsequent to internalization in the nuclear translocation process. Because angiogenin activates PLC activity in endothelial cells (11) and PLC activity in turn is needed for nuclear translocation, the two cellular events may be interrelated and cooperate for the ultimate activity of angiogenin in endothelial cells. It is known that several cellular signal pathways activated by ligands binding to their receptors often crosstalk to obtain optimal cellular function (29, 30).

Genistein, oxophenylarsine and staurosporine, which inhibit tyrosine kinase, phosphotyrosine phosphatase and protein kinase C, respectively, do not inhibit nuclear translocation of angiogenin. At present, it is unknown whether or not they inhibit angiogenin-induced proliferation and angiogenesis. If they do, the mechanisms would be different from that by which neomycin exerts its anti-angiogenesis effects.

Several compounds of microbial origin have been reported to inhibit endothelial cell function. These include anthracycline (31), 15-deoxyspergulin (32), D-penicillamine (33), eponemycin (34), fumagillin (35), herbimycin (36), and rapamycin (37). The mechanism by which these antibiotics inhibit endothelial cells or in some cases, angiogenesis, are unknown.

The present results indicate that neomycin inhibits angiogenin-induced angiogenesis, mainly through its inhibition of

nuclear translocation of angiogenin in endothelial cells. Because neomycin targets intracellular events, it will not encounter the difficulties associated with the circulating angiogenin in plasma when it is to be developed as an anti-angiogenin agent. The data suggest that neomycin and its analogs may represent a new class of compounds with therapeutic potential for the clinical treatment of angiogenesis-related diseases.

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